Ecotoxicity of Silver Nanoparticles

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By

Lauren Corrigan

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The Ohio State University

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Honors Thesis Examination Committee:

Dr. John J. Lenhart, Advisor

Dr. Harold W. Walker

Approved By:

John Lenhart
Advisor
Undergraduate Program in
Engineering

ABSTRACT

Due to the recent advances in nanotechnology, nanoparticles are becoming more prevalent in society. However, the impact on the environment as a result of these nanoparticles remains largely unknown. The purpose of this research is to provide some insight regarding the ability of nanosilver to produce reactive oxygen species (ROS), which are believed toxic. The production of ROS, hydrogen peroxide in particular, was studied as a function of the composition of water, more specifically the pH of the water. A suspension of silver nanoparticles was synthesized and the concentration of hydrogen peroxide was measured using a fluorometer for nanosilver suspensions at pH values of 4, 7, and 10. Along with the hydrogen peroxide production, the characterization of the nanosilver suspension was also determined with respect to size and stability. The zeta potential and the hydrogen peroxide production were determined to be a function of the pH of the suspension. With regard to the zeta potential, the magnitude of the zeta potential is at its maximum around the neutral pH value and the zeta potential value becomes less negative and closer to zero as the acidity of the suspension increase. The initial hydrogen peroxide rate also varied with respect to the pH. The initial rate of hydrogen peroxide production was observed to decrease with an increase in pH. Further reproduction of the hydrogen peroxide experiments are required, however, in order to arrive at any concrete conclusions.

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VITA

2006 – Present……Undergraduate Student,
At The Ohio State University

2009 – Present……Undergraduate Researcher,
At The Ohio State University

PUBLICATIONS

Not Applicable

FIELDS OF STUDY

Major Field: Civil Engineering

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CHAPTER 1

INTRODUCTION

Nanotechnology involves creating, manufacturing, and manipulating materials at a scale that falls within the range of 1 to 100 nanometers. Nanoscale materials, also known as nanoparticles, have the potential to produce revolutionary advances in medicine through targeted drug delivery and electronics based upon their size-dependent optical properties (US EPA 2008). Nanotechnology is also being applied to issues involving the environment and agriculture (US EPA 2008). The use of nanotechnology in both public and private sectors is rapidly increasing and is expected to continue increasing in the future. Figure 1 indicates the expected growth of various nanotechnologies between 2007 and 2013. Figure 1 shows that nanomaterials account for the greatest percent of the global nanotechnology market by 2013. Unavoidably, the manufacture and use of nanoparticles will lead to their presence in the environment and the potential risks to ecosystem and human health remain essentially unknown (Wiesner 2006). Due, in part, to their exceptionally small size, nanoparticles have a number of unique properties that make them so commercially desirable. For example, their small size also produces large surface areas and as a result, they tend to be more reactive than the same material at a larger scale. One adverse consequence of their exceptional reactivity is that nanoparticles could prove to be more toxic to the environment than their larger scale counterparts (Choi 2008).

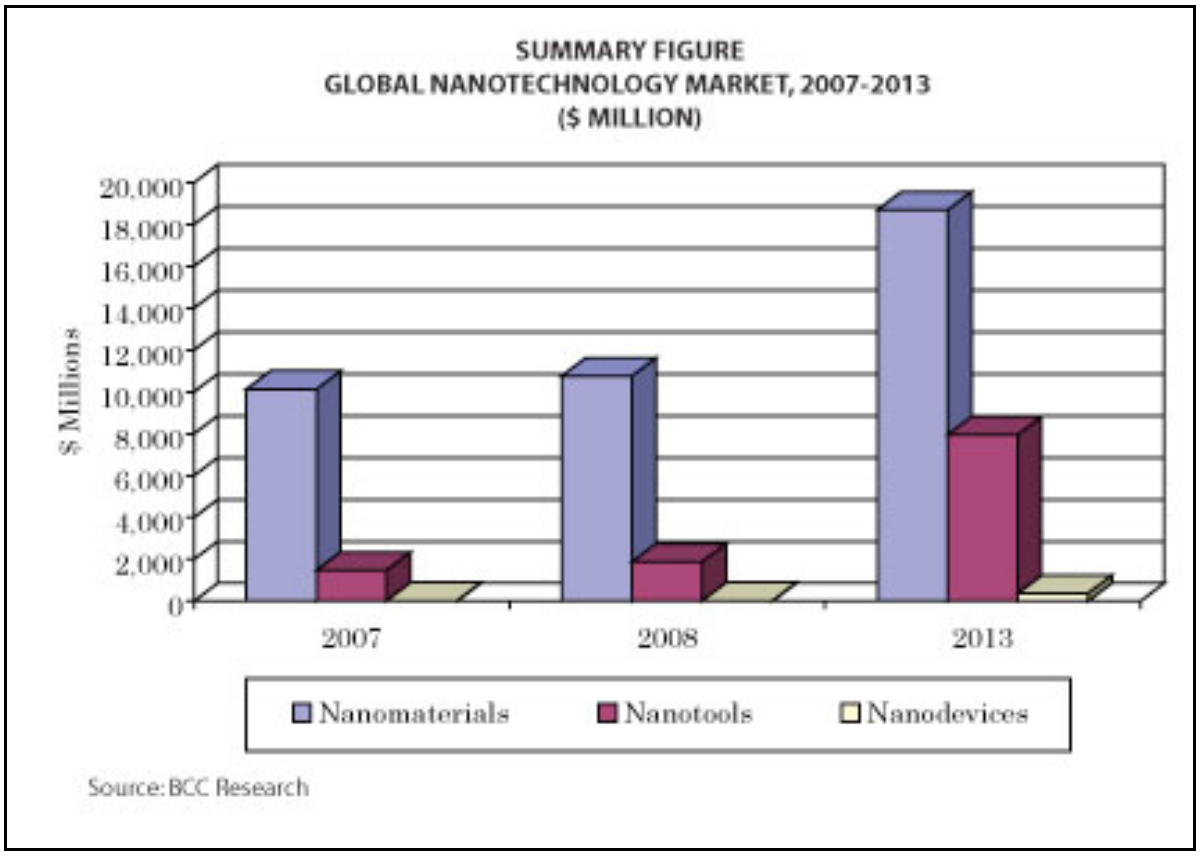

Figure 1 – Expected growth of the global nanotechnology market over the period from 2007 to 2013. Taken from McWilliams (2008)

Silver is widely known for its antimicrobial qualities and thus it is utilized quite frequently in areas of medicine. The effectiveness of nanosilver as an antimicrobial agent is evident in Figure 2 by the decrease in the number of bacterial colonies with the increase in nanosilver concentration. The effect of the nanosilver on the number of bacterial colonies is similar to that of amoxicillin, which can be observed in Figure 3. Due to the antimicrobial property of silver, nanosilver is among the most commonly used nanoparticles (Choi 2008). For example, silver nanoparticles were introduced in antiseptic ointments for wound care in 1990 when it was determined how effective nanosilver was in killing bacteria when the ointment was applied to wounds (Arora 2008).

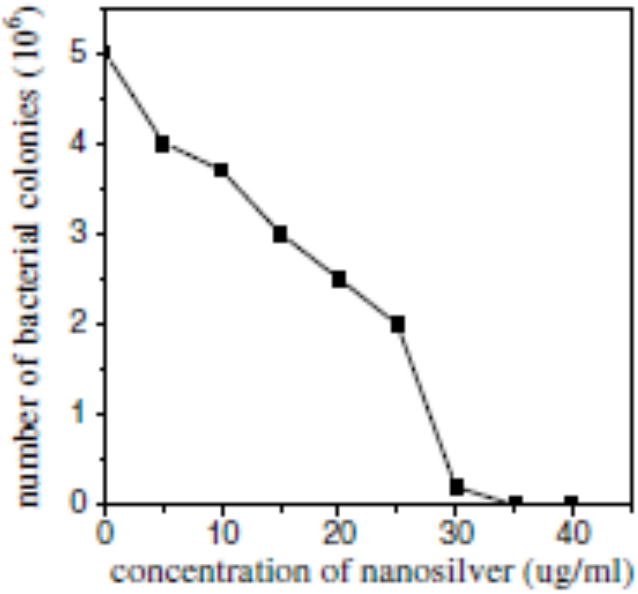

Figure 2 – The effect of different concentrations of nanosilver on 5 X 10^6 CFU of bacterial colonies. Taken from Li et al. (2005).

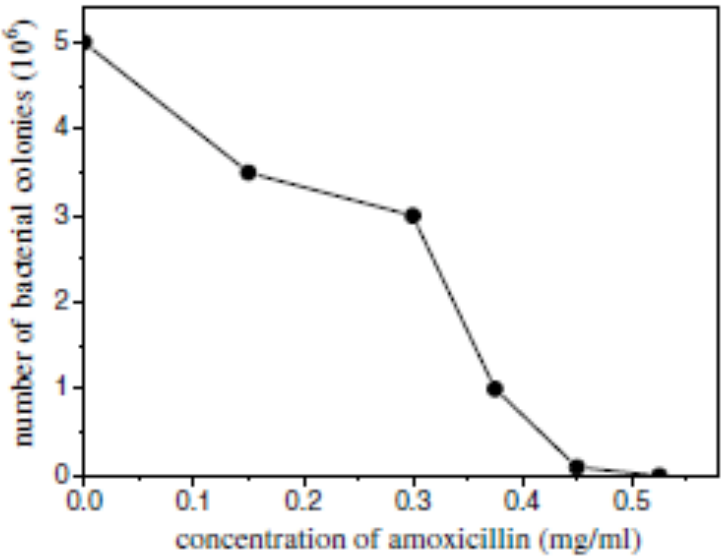

Figure 3 – The effect of different concentrations of amoxicillin on 5 X 10^6 CFU of bacterial colonies. Taken from Li et al. (2005).

In addition to the use of nanosilver in medical applications, it has also been used in household appliances due to its antimicrobial properties. The company, Samsung, uses nanosilver in its washing machines to remove odor-causing bacteria (Binion 2008). However, the same antimicrobial properties that make silver nanoparticles so beneficial to the medical field could prove harmful when found in ecosystems. Microorganisms are essential to the environment. Aiding in nitrogen fixation and other nutrient cycles, as well as decomposing organic matter are just a couple of their important roles (Wiesner 2006). Further research regarding nanosilver is necessary due to the crucial role microorganisms have in the environment and the insufficient understanding of the risks caused by silver nanoparticles to microbial ecology.

In order to recognize and gauge the potential risks created by silver nanoparticles, it is imperative to understand the behavior and expected concentrations of these

nanoparticles in the surrounding environment (Colvin 2003). Once it is introduced into natural water, the possible toxicity of nanosilver poses a threat to any aquatic life found in that water and any organism depending on that water to survive. For instance, zebrafish are among a variety of aquatic species for which nanosilver has been observed to be toxic (Griffitt 2008). In the presence of toxins, normal metabolic processes that regulate reactive oxygen species (ROS) accumulation are disrupted, causing the cells to accumulate excess ROS, which can lead to cell death. Destructive ROS include superoxide (O_2-), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2). Hydrogen peroxide is of particular concern with regard to silver nanoparticles since studies have detected its presence in nanosilver suspensions (Liu 2010). While experiments show that ROS, particularly hydrogen peroxide, can amass in cells when in the presence of silver nanoparticles, the origin of the ROS in relation to nanosilver is largely unknown (Choi 2008). However, recent studies reveal that hydrogen peroxide production is associated with the nanosilver as opposed to silver ions (Liu 2010). Therefore, the focus of this research will center around how ROS production from the silver nanoparticles depends on the composition of water, specifically the pH.

CHAPTER 2

EXPERIMENTAL METHODS

2.1 Overview

The research was conducted in a series of three tasks. In order to understand how silver nanoparticles behave in nature, it was necessary to identify their characteristic physical and chemical properties. Therefore once the silver nanoparticles were synthesized, the particle size, shape, and surface potential were measured. Finally, the ROS (in the form of hydrogen peroxide) generated from the nanosilver was measured as a function of the pH and time. The equipment required to complete the research is located in the environmental engineering research laboratory and other areas located on campus.

Task 1 involved synthesizing the silver nanoparticles. The nanosilver was prepared through the chemical reduction of $[Ag(NH_3)_2]^+$ by D(+)-maltose following the procedure of Panacek et al. (2006). This procedure produces particles ranging in size from 60 nm to 90 nm and the chemicals required for the reaction include silver nitrate, ammonia, and D-glucose. At the conclusion of the synthesis, the non-reacted chemicals will be removed from the particle suspension using dialysis tubing.

Task 2 entailed characterizing the physical and chemical properties of the silver nanoparticles. This was necessary to better understand whether the particles will remain suspended in water or whether they may aggregate and potentially settle out of the water. The average hydraulic radius of the nanosilver was obtained using a Zeta Plus Particle Size Analyzer (Brookhaven Instr. Co.). The surface potential of the silver nanoparticles was also indirectly characterized based on their zeta potential, which was measured with

the same Zeta Plus Particle Size Analyzer used to determine the diameter of the nanosilver.

Task 3 included measuring the ROS generated from the silver nanoparticles as a function of the pH. The pH was adjusted using nitric acid (or sodium hydroxide). The amount of ROS was determined from measuring the quantity of hydrogen peroxide (H_2O_2). The H_2O_2 was measured using a fluorometer. In order to be detected by spectrofluorecence, horseradish peroxidase was applied to catalyze the reaction between hydrogen peroxide and p-hydroxylphenylacetic acid. A slit width of 1.5 nm was used to detect the fluorescence at an excitation (EX) wavelength of 320 nm and an emission (EM) wavelength of 400 nm (Kosaka 1998). These measurements were conducted using nanosilver with pH values ranging from 4 to 10.

2.2 Synthesis of Silver Nanoparticles

Before beginning the synthesis of the nanosilver, all glassware was soaked in a 10% nitric acid wash overnight, rinsed with deionized water, and oven-dried under dust-free conditions. The synthesis of the silver nanoparticles required four solutions of the following reaction components as stated in Panacek et al. (2006): ammonia, D-maltose, silver nitrate, and sodium hydroxide. The concentrations were 0.02 M, 0.025 M, 0.01 M, and 0.1 M for the ammonia, D-maltose, silver nitrate, and sodium hydroxide, respectively. The four solutions were filtered with using a 100 nm cellulose acetate membrane. The actual synthesis process consisted of 4 mL of the silver nitrate solution that was pipetted into a 50 mL glass beaker. Then 20 mL of the ammonia solution was added to the beaker and a small, magnetic stirring bar started stirring the solution at a low

speed. Following the addition of the ammonia, 500 μL of the sodium hydroxide solution was added to adjust the pH of the solution to approximately 11.5. Lastly, 16 mL D(+)-maltose was added to reduce the $[Ag(NH_3)_2]^+$ that had formed into the silver nanoparticles. This process lasted approximately 20 minutes in an environment devoid of light.

Immediately following the synthesis, the nanosilver suspension was dialyzed to remove any non-reacted residuals. The type of dialysis tubing used consisted of cellulose ester membranes (Spectra/Por® Biotech) with a molecular weight cut off (MWCO) of 8-10 k Daltons. Before using the dialysis tubing, it was soaked in deionized water for 15 minutes in order to remove the preservative agent, sodium azide. After soaking the dialysis membrane and thoroughly rinsing it with deionized water, the suspension of freshly synthesized silver nanoparticles was poured into it and clamped shut with two closures. The full dialysis tubing was then placed into a 2 L glass beaker, which was filled with deionized water and contained a large, magnetic stirring bar used to stir the water during the dialysis process. The silver nanoparticle suspension was dialyzed at room temperature, in a dark environment, for 12-20 hours with three complete changes of the deionized water to ensure the complete removal of residuals.

2.3 Characterization of Silver Nanoparticles

In order to ascertain the potential threat posed by the presence of silver nanoparticles in the environment, it was necessary to determine the specific characteristics and properties that have an impact on the fate of the nanoparticles when they enter the environment. The necessary characterization was accomplished through

determining the zeta potential, size and UV absorbance of the silver nanoparticles. The zeta potential (ζ) is a measure of the electrostatic potential on the surface of the nanoparticle and is related to the electrophoretic mobility and stability of the suspension of nanoparticles (Dougherty, 2007). The zeta potential of the nanosilver suspension was determined using a Zeta Plus Particle Size Analyzer (Brookhaven Instr. Co.). This same instrument was also used to determine the average effective diameter of the silver nanoparticles (Soukupova 2008). In order to obtain both zeta potential and effective diameter measurements of the silver nanoparticles, 1 mL of the nanosilver suspension was diluted to 10 mL with deionized water, which was then pipetted into a cuvette and then placed in the Zeta Plus Particle Size Analyzer.

The UV absorbance of the silver nanoparticles is another measurement that can aid in the characterization of the nanosilver suspension. A UV/VIS spectrophotometer (Shimadzu UV-2401 PC) was the instrument used to obtain the UV absorbance measurements.

2.4 H_2O_2 Production

Before beginning work on the fluorometer (RF-5301 PC, Schimadzu), the reagent for the H_2O_2 RF measurement had to be prepared. The reagent is a mixture of 0.15 M p-hydroxyphenylacetic acid, 1.5 units of peroxidase per mL of solution, 0.26 M formaldehyde, 0.005 M EDTA, and 0.5 M tris. The purpose of the formaldehyde and the EDTA is to reduce the interference of hydroxymethanesulfonate and metals, respectively (Kok 1986). The tris was used as a buffer to increase and stabilize the pH of the solution to a pH value between 8 and 9. A strong base, 5 N NaOH, was added to further adjust

the pH of the solution to a pH value of 9. When the reagent used to measure the hydrogen peroxide was not in use, it was covered in tin foil and stored in the refrigerator.

In order to analyze the hydrogen peroxide production from the silver nanoparticles, a standard curve was first created using hydrogen peroxide solutions of varying concentrations. Concentrations of 0.5, 1, 2, 4, 6, and 10 μM H_2O_2 solution was created from diluting 30% (v/v) H_2O_2 stock solution. The H_2O_2 reagent was added in approximately a 1:25 ratio to the standard that was about to be measured in the fluorometer. The sample and the reagent were allowed to react for 5 minutes before being measured in the fluorometer. The average value of intensity detected by spectrofluorecence for each standard was calculated to generate the working curve.

Once all of the standards were measured in the fluorometer, the hydrogen peroxide production from the silver nanoparticles was determined. The dialyzed nanosilver suspension was first divided into 3 separate beakers, each beaker containing 12 mL of the nanosilver suspension diluted to 50 mL with deionized water. Using 0.2 N HCl and 0.2 N NaOH, the pH of the samples of silver nanoparticles was adjusted such that the pH of one sample was approximately 4, the pH of another sample was approximately 7, and the pH of the final sample was approximately 10. 3 mL of the nanosilver suspension at each pH value was filtered using a 0.020 μm glass fiber filter and the initial concentration of H_2O_2 in the filtrate was measured in the fluorometer employing the same techniques used when determining the standard curve. After determining the initial hydrogen peroxide concentration in the samples of the nanosilver suspension, they were left in the presence of light and measurements were taken over the

course of 10 hours following the same method used to determine initial H_2O_2 concentrations.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Suspension Synthesis and Characterization

A few problems were encountered while synthesizing the nanosilver suspension. The most notable problem occurred after using a glass fiber filter as opposed to a cellulose acetate membrane to filter the various reagents used to make the silver nanoparticles. When synthesized properly, the nanosilver suspension is a deep, vibrant orange color, which is shown in Figure 4; however, as a result of using the glass fiber filter, the suspension of silver nanoparticles turned green-purple in color after dialysis.

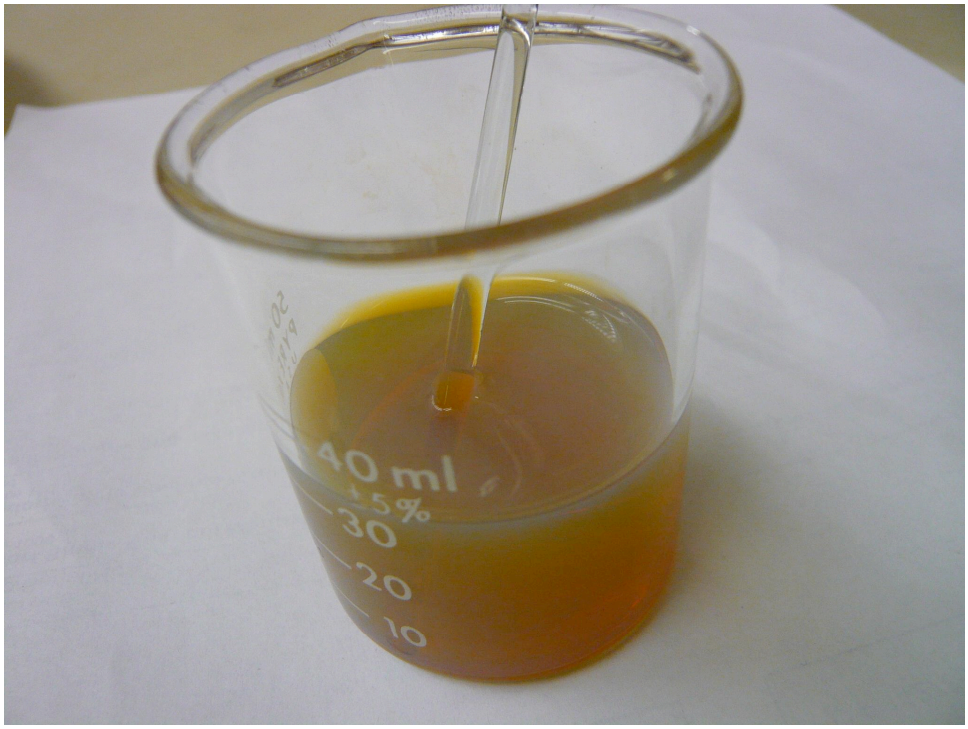

Figure 4 – Image of correctly synthesized nanosilver suspension

The discolored suspension was analyzed with regard to size and it was determined that the polydispersity of this particular batch of silver nanoparticles was unusually high. The average effective diameter and polydispersity of the discolored silver nanoparticle suspension are shown in Table 1. The cellulose acetate membrane was used to filter the reagents in subsequent syntheses to avoid this problem. Other problems that were encountered when synthesizing the nanosilver suspension, which resulted in highly polydispered suspensions, were due to using expired chemicals for the synthesis. These problems were remedied by ordering new chemical reagents, which is supported by the lower polydispersity of the nanosilver suspension observed in Table 2. This table shows the size characterization for the nanosilver suspension used in the analysis of the hydrogen peroxide production. The size and polydispersity data for different batches of nanosilver suspension prepared on different occasions is included in the Appendix.

Table 1 – Characterization of green-purple nanosilver suspension

Size Characterization	
Average Effective Diameter (nm)	84.5
Average Polydispersity	0.293

Table 2 – Characterization of nanosilver suspension used in hydrogen peroxide production experiment

Size Characterization	
Average Effective Diameter (nm)	68.0
Average Polydispersity	0.137

As a result of determining the zeta potential of the nanosilver suspension, the likelihood of whether or not the silver nanoparticles will aggregate and potentially settle out of suspension was ascertained. The higher the absolute value of the zeta potential, the more likely the nanoparticles will remain in suspension; whereas conversely, the lower the magnitude of the zeta potential, the more likely the nanoparticles will aggregate together (Dougherty 2007). The zeta potential of the nanosilver suspension at various pH values corresponding to a specific electrolyte concentration of 1 mM NaCl is shown in Figure 5. These results are consistent with the findings by Dougherty et al. (2007). Dougherty et al. (2007) observed all negative zeta potential values for silver nanoparticles and found that the magnitude of the zeta potential was "typically maximized at pH values in the neutral or slightly basic range" and that the zeta potential values approached zero "toward the acid end of the range." This same trend is observed in Figure 5; the magnitude of the zeta potential is at its maximum around the neutral pH value and the zeta potential value becomes less negative and closer to zero as the acidity of the suspension increase. The maximum zeta potential magnitude for the nanosilver suspension as observed in Figure 5 is approximately -50 mV.

The zeta potential also indicates the presence of an oxidized surface layer. If there is no oxide-layer on the surface of the silver nanoparticles, the surface charge should theoretically be zero. Due to the negative potential observed in Figure 5, an oxidized surface layer is likely present on the silver nanoparticles (Li et al. 2010). The UV-VIS absorption spectrum was determined to verify this conclusion.

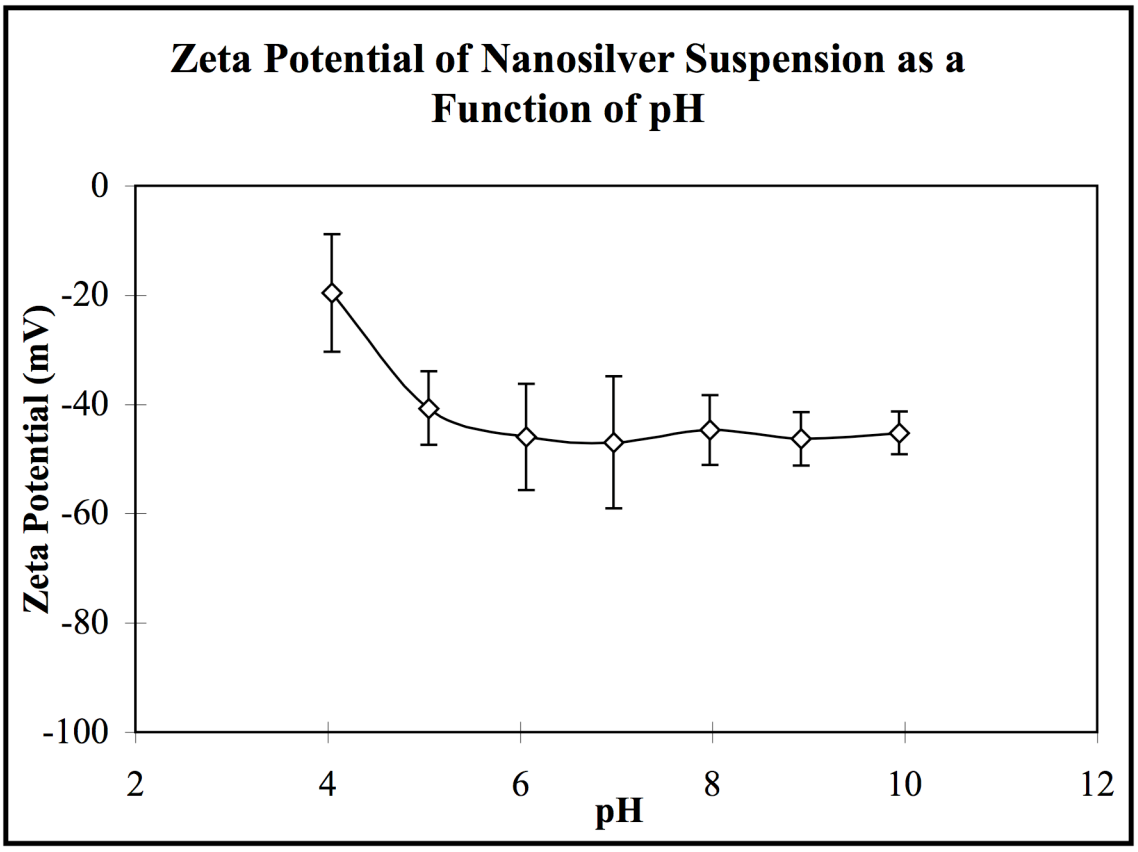

Figure 5 – The zeta potential of nanosilver suspension as a function of pH. Sample tested at an electrolyte concentration of 1 mM NaCl. Taken from Li et al. (2010)

Measuring the UV absorbance of the nanosilver suspension provided another method of characterization. The UV absorbance provides information regarding the size and structure of the nanoparticles based on the surface plasmon resonance of the nanoparticles (Elechiguerra 2005). The UV absorbance of the nanosilver suspension can be observed in Figure 6, which has a surface absorption peak at a wavelength of approximately 440 nm. Panacek et al. (2006) observed absorption peaks between wavelengths of 390 – 420 nm for silver nanoparticles ranging in size between 25 – 50 nm. Since the wavelengths of the surface plasmon resonance peak increases with

increasing particle size, a peak at approximately 440 nm is reasonable for silver nanoparticles ranging between effective diameters of 65 to 85 nm (Elechiguerra, 2005).

The UV absorbance also provides an indication of the presence of an oxidized surface layer or absorbed substances. If neither is present, the absorption peak is narrow and located at a wavelength ranging between 390 nm and 450 nm. For this experiment, an oxidized layer on the nanoparticles is likely since there was no attempt to control the atmosphere during synthesis or dialysis (Li et al. 2010). The presence of an oxidized surface layer is supported by the red-shift in peak location as well as the broader absorption peak, which is observed in Figure 6.

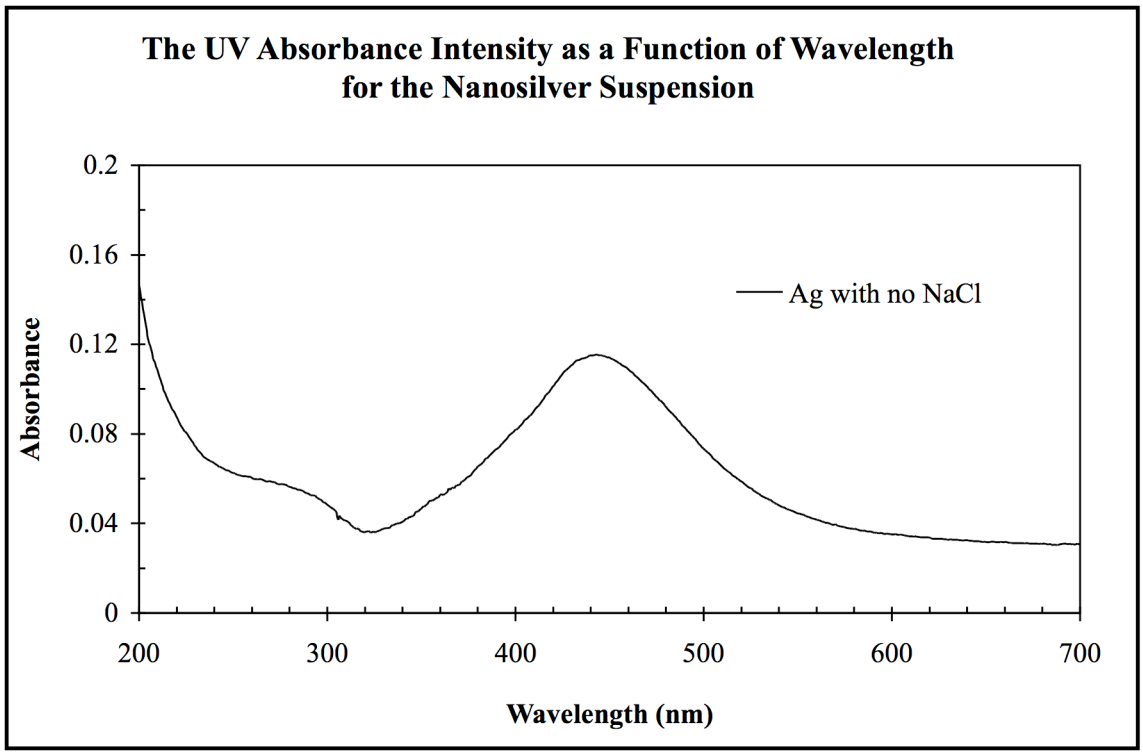

Figure 6 – UV absorbance spectrum for nanosilver suspension. Taken from Li et al. (2010)

Since the shape of the particle also affects the surface plasmon resonance, the UV absorbance of the nanosilver suspension was used to determine the shape of the silver nanoparticles as well as the size. Through comparing UV absorption diagrams and TEM images of silver nanoparticles, Mock et al. (2002) determined the relationship between the surface plasmon resonance peak and the shape of the silver nanoparticles. For instance, silver nanoparticles with a peak around a wavelength of approximately 625 nm were triangular in shape, whereas particles with a peak around a wavelength of approximately 450 nm were spherical in shape (Mock 2002). Thus, it can be deduced that the nanosilver suspension used for the hydrogen peroxide analysis was spherical in shape due to the UV absorption peak around a wavelength of approximately 440 nm.

3.2 H_2O_2 Production

As discussed in the H_2O_2 Production Methodology section above, six standards of varying concentrations of hydrogen peroxide were created to provide a standard curve, which enabled the quantification of the hydrogen peroxide production from the nanosilver suspension. The hydrogen peroxide standard curve is shown in Figure 7. The correlation between the hydrogen peroxide concentration and the fluorescence value was observed to have a relatively strong linear relationship; thus a best-fit linear regression line was created corresponding to the data, which was used to determine the concentration of hydrogen peroxide produced from the suspension of silver nanoparticles. The linear correlation data for the hydrogen peroxide standard curve is shown in Table 3. The amount of hydrogen peroxide produced from the suspensions of silver nanoparticles at various pH values was determined from following the procedure described in the H_2O_2

Production Methodology section. The hydrogen peroxide measurements over the course of ten hours are observed in Figure 8 for each nanosilver suspension of a specific pH value.

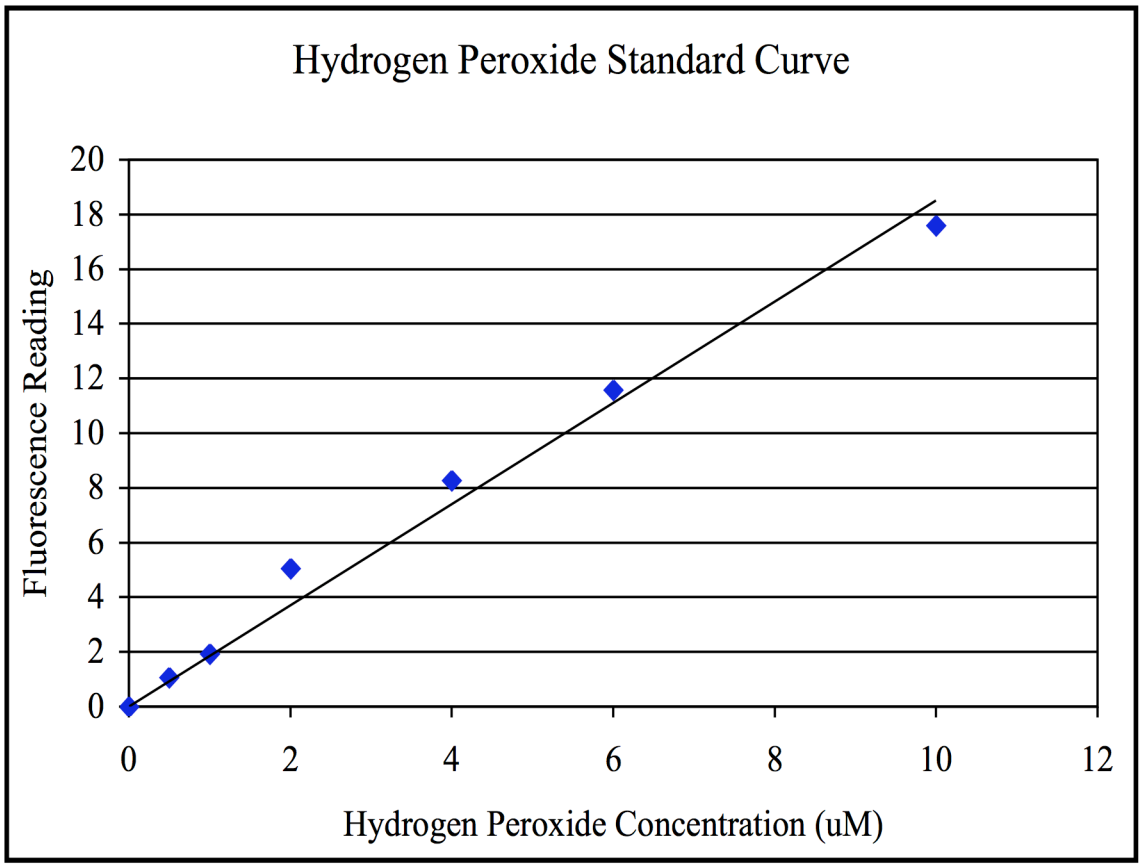

Figure 7 – Hydrogen Peroxide Standard Curve that shows the relationship between the fluorescence reading and hydrogen peroxide concentration

Table 3 – Linear Correlation data for the hydrogen peroxide standard curve

Linear Correlation Data	
Linear Regression Line	y=1.8508x
Coefficient of Determination	R^2=0.9851

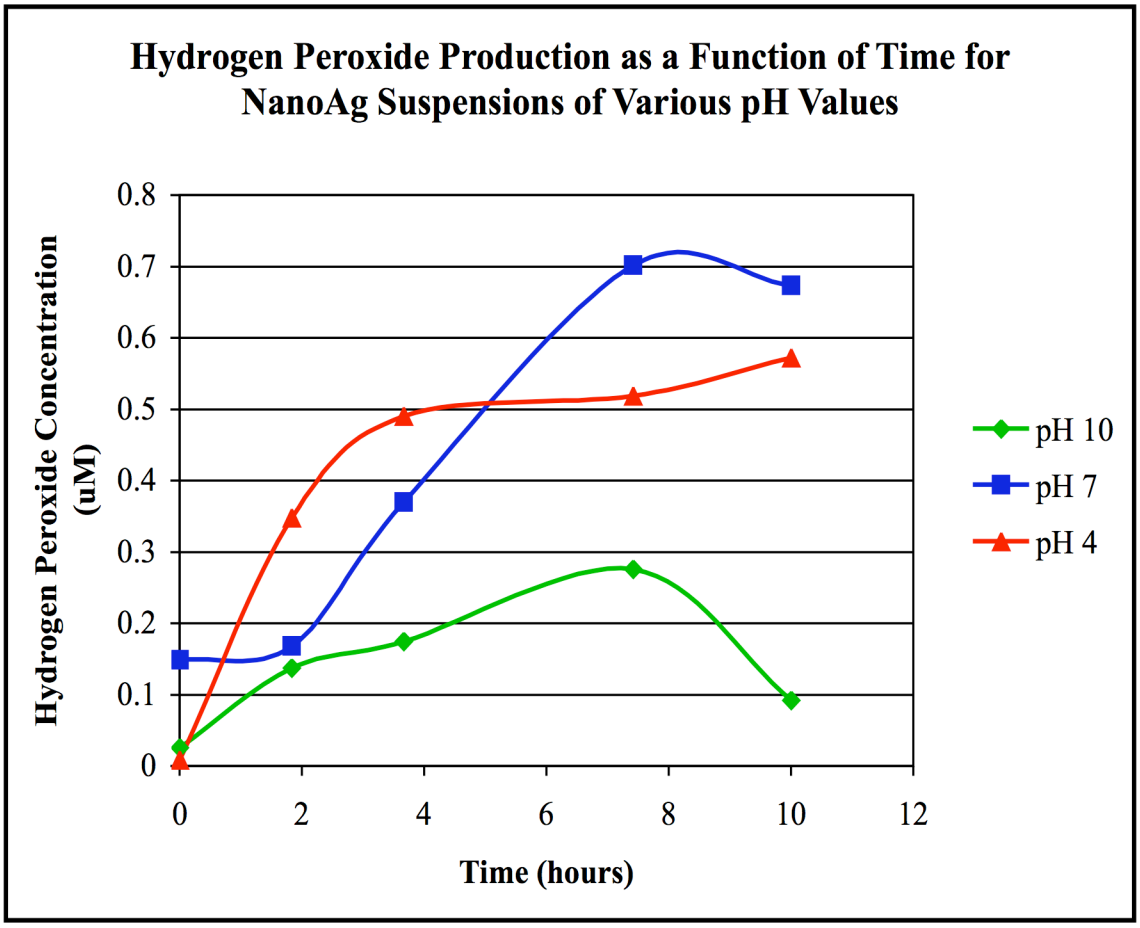

Figure 8 – The hydrogen peroxide production for nanosilver suspensions of various pH values taken over the course of 10 hours

As evident from Figure 8, the hydrogen peroxide production varies with respect to time and the pH of the suspension. Regardless of pH, the general trend in the hydrogen peroxide production for each of the nanosilver suspensions is similar. The hydrogen peroxide concentration initially increases with time, and then eventually starts to level out. The only noticeable difference in the general trend of hydrogen peroxide production between the suspensions of differing pH values is that the hydrogen peroxide

concentrations start to decrease for the nanosilver suspensions of pH 7 and pH 10 after approximately 8 hours of light exposure. The decrease in hydrogen peroxide concentrations can be associated with its high reactivity (Choi 2008).

Based on Figure 8, one significant difference in the hydrogen peroxide production as a result of the pH of the suspension is the magnitude of the hydrogen peroxide concentration. The pH 7 nanosilver suspension produced the highest concentration of hydrogen peroxide, with a peak of approximately 0.70 μM. The pH 10 nanosilver suspension, on the other hand, produced the lowest concentration of hydrogen peroxide. The pH 10 suspension had a peak hydrogen peroxide concentration of approximately 0.28 μM.

Figure 8 also indicates that the initial rate of hydrogen peroxide production appears to vary with respect to the pH of the suspension. The general trend in the initial hydrogen peroxide rate with respect to the pH is observed in Figure 8 after disregarding the initial hydrogen peroxide concentration of the pH 7 suspension since it is likely an error since it does not follow the same general trend exhibited by the other data points. In actuality, the initial hydrogen peroxide concentration for the pH 7 suspension is likely closer to the initial hydrogen peroxide concentrations of the pH 4 and pH 10 nanosilver suspensions. Taking the possible error into consideration, the initial hydrogen peroxide rate appears to decrease with increasing pH: the initial hydrogen peroxide production rate is the greatest for the pH 4 nanosilver suspension and it is the least for the pH 10 nanosilver suspension.

In order to assess whether the hydrogen peroxide production was a result of the of the nanosilver suspension or strictly the pH change, the hydrogen peroxide concentrations

of deionized water at the same pH values as the nanosilver suspensions were analyzed. The pH of the deionized water was adjusted to the specific values using HNO_3 and NaOH, just as what was done with the suspensions of silver nanoparticles. After about six hours of light exposure, the deionized water samples were analyzed and the hydrogen peroxide concentrations can be observed in Figure 9. Due to the low concentrations of hydrogen peroxide observed in Figure 9 for deionized water at pH values of 4, 7, and 10, the effect of the pH adjustment on the hydrogen peroxide production can be assumed as negligible. In addition to determining whether the pH adjustment was alone responsible for the hydrogen peroxide production or whether it was associated with the silver nanoparticles, the source of the hydrogen peroxide was also analyzed; specifically, whether its production was due to the silver ions or the silver nanoparticles in particular. A study conducted in 2010 by Liu and Hurt examined the hydrogen peroxide production from a nanosilver suspension and from a solution containing $AgClO_4$ (2 mg/L Ag+). The study revealed that the $AgClO_4$ solution produced no detectable amount of hydrogen peroxide; whereas, the suspension of silver nanoparticles produced a hydrogen peroxide concentration of approximately 0.4 μM. Therefore, based on this study, it can be concluded that the hydrogen peroxide production is indeed associated specifically with the silver nanoparticles as opposed to silver ions.

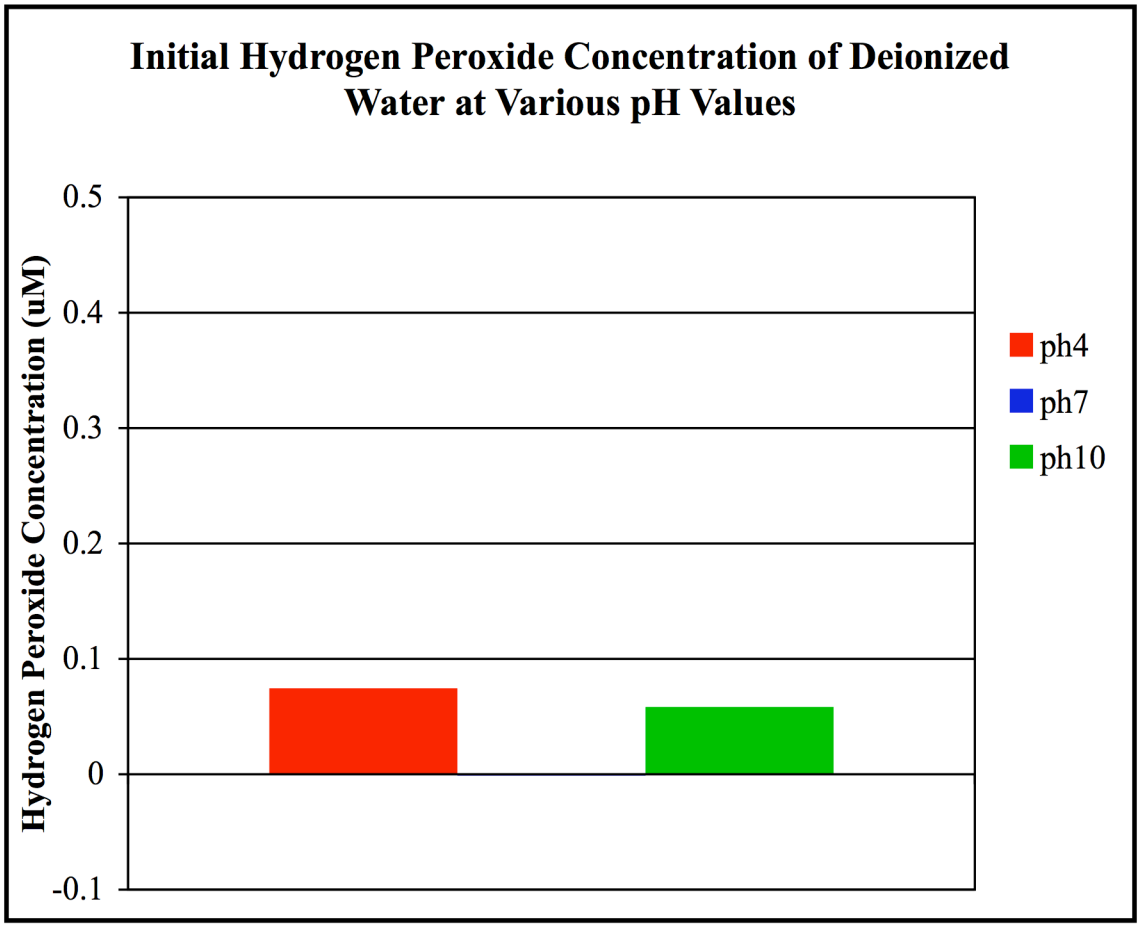

Figure 9 – The initial hydrogen peroxide concentration for various pH values of deionized water

3.3 Reproduction of Experiments

In order to make any significant conclusions, reproduction of the experiment is essential. The synthesis of the nanosilver suspension was reproduced on multiple occasions, most of which yielded similar characteristics. The hydrogen peroxide production experiment, however, was only conducted once with fresh reagents; therefore, further reproductions are necessary in order to ascertain the accuracy of the data. Data

from an initial attempt to determine the hydrogen peroxide production from nanosilver suspension at a pH value of 4 is included in the Appendix.

CHAPTER 4

CONCLUSION

The potential risks posed by the presence of silver nanoparticles in the environment can be gauged in a variety of ways. For instance, the characteristics of the silver nanoparticles such as size, stability, and mobility provides information pertaining to the potential risks. The small size of the silver nanoparticles amounts to a large surface area and as a result, they tend to be highly reactive. The higher reactivity of the silver nanoparticles compared to that of the same material on a larger scale increases the toxic effect to the environment (Choi 2008). The stability of the nanosilver suspension also has an impact on the potential risks. The more stable the nanosilver suspension, the greater the risk is to the environment. The toxic effect from the silver nanoparticles could potentially impact a greater surrounding area as long as the nanoparticles remain in suspension and remain mobile. Based on the zeta potential results, observed in Figure 5, the nanosilver suspension is more likely to remain stable at higher pH values than the nanosilver suspension at lower pH values due to the higher zeta potential magnitudes associated with the former suspension. At lower pH values, the zeta potential magnitude is closer to zero; therefore, there is a greater chance that the nanoparticles will aggregate together and potentially settle out of the surrounding fluid.

The zeta potential as well as the hydrogen peroxide production depends on the pH of the nanosilver suspension. Based on Figure 8, the nanosilver suspension at a neutral pH value produces the maximum concentration of hydrogen peroxide. This also coincides with pH value at which the nanosilver suspension is most stable. The higher

particle stability could adversely impact aquatic organisms. The nanosilver suspension at pH 4 also produces a fair amount of hydrogen peroxide over the course of ten hours; however, it likely poses less of a threat to aquatic organisms since the particles are more likely to settle out of the water due to the instability of the suspension determined from the zeta potential. The initial hydrogen peroxide rate also varied with respect to the pH. The initial rate of hydrogen peroxide production was observed to decrease with an increase in pH. Figure 8 shows that the pH 4 nanosilver suspension had the greatest initial rate of hydrogen peroxide production, whereas the pH 10 nanosilver suspension had the lowest initial rate of hydrogen peroxide production. Further reproduction of the hydrogen peroxide experiments are required, however, in order fully support these conclusions.

APPENDIX

Table 4 – The size characterization for the nanosilver suspension synthesized on January 19th, 2010

Size Characterization		
Trial 1	Average Effective Diameter (nm)	84.2
	Average Polydispersity	0.256
Trial 2	Average Effective Diameter (nm)	86.6
	Average Polydispersity	0.264
Average	Average Effective Diameter (nm)	85.4
	Average Polydispersity	0.26

Table 5 – The size characterization for the nanosilver suspension synthesized on February 24th, 2010

Size Characterization	
Average Effective Diameter (nm)	56.2
Average Polydispersity	0.068

Table 6 – The size characterization for the nanosilver suspension synthesized on March 4th, 2010

Size Characterization		
Trial 1	Average Effective Diameter (nm)	63.9
	Average Polydispersity	0.169
Trial 2	Average Effective Diameter (nm)	65
	Average Polydispersity	0.201
Average	Average Effective Diameter (nm)	64.45
	Average Polydispersity	0.185

Table 7 – The size characterization for the nanosilver suspension synthesized on April 5th, 2010

Size Characterization	
Average Effective Diameter (nm)	66.8
Average Polydispersity	0.144

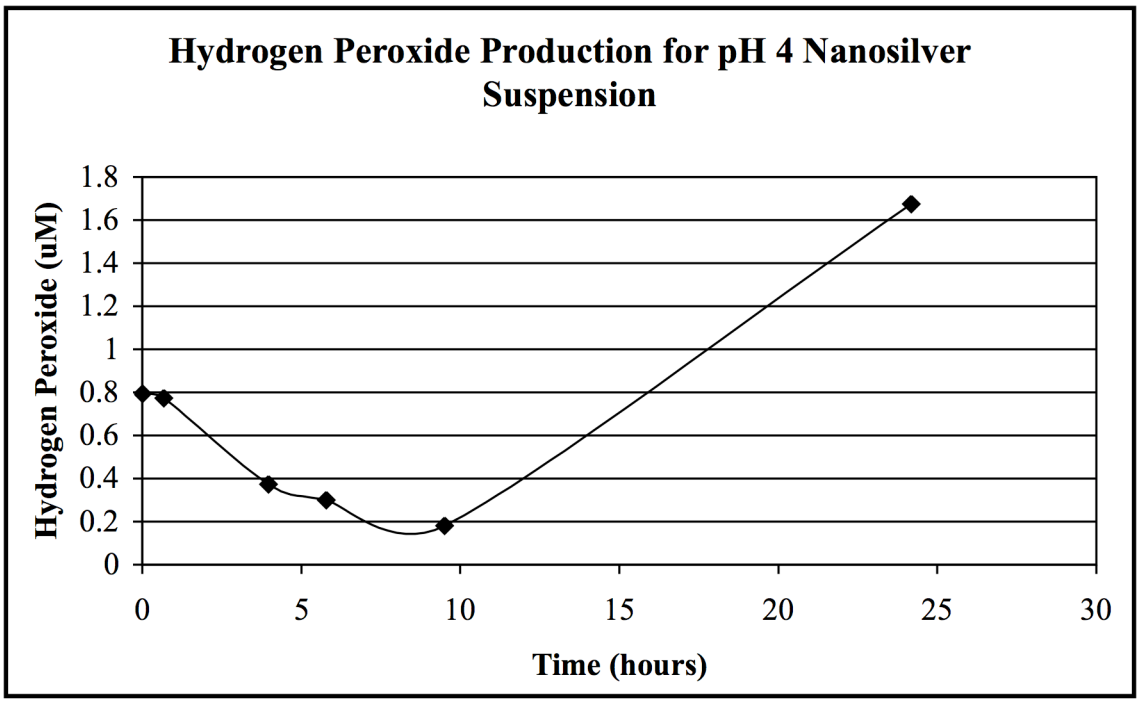

Figure 10 – The hydrogen peroxide production for a pH 4 nanosilver suspension from an experiment conducted on March 5th, 2010.

LIST OF REFERENCES

Ahamed, M., M. Karns, et al. (2008). "DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells." Toxicology and Applied Pharmacology 233: 404-410.

Arora, S., J. Jain, et al. (2008). "Cellular responses induced by silver nanoparticles: In vitro studies." Toxicology Letters 179: 93-100.

Binion, Robert. (2008). "Nanotechnology: A little bit like asbestos?" Mealey's Litigation Report: Insurance 22(28): 1-5.

Carlson, C., S.M. Hussain, et al. (2008). "Unique Cellular Interaction of Silver Nanoparticles: Size-Dependent Generation of Reactive Oxygen Species." The Journal of Physical Chemistry B 112: 13608-13619.

Choi, O. and Z. Hu (2008). "Size Dependent and Reactive Oxygen Species Related to Nanosilver Toxicity to Nitrifying Bacteria." Environmental Science and Technology 42(12): 4583-4588.

Colvin, V.L. (2003). "The potential environmental impact of engineered nanomaterials." Nature Biotechnology 21(10): 1166-1170.

Dougherty, Geroge M., K.A. Rose, et al. (2007). "The zeta potential of surface-functionalized metallic nanorod particles in aqueous solution." Electrophoresis 29: 1131-1139.

Elechiguerra, J.L., et al. (2005). "Interaction of silver nanoparticles with HIV-1." Journal of Nanobiotechnology 3(6): 1-10.

Griffitt, R.J., K. Hyndman, et al. (2008). "Comparison of Molecular and Histological Changes in Zebrefish Gills Exposed to Metallic Nanoparticles." Toxicological Sciences 107(2): 404-415.

Klaine, S.J., P.J. Alvarez, et al. (2008). "Nanomaterials in the Environment: Behavior, Fate, Bioavailability, and Effects." Environmental Toxicology and Chemistry 27: 1825-1851.

Kok, Gregory L., K. Thompson, et al. (1986). "Derivatization Technique for the Determination of Peroxides in Precipitation." Analytical Chemistry 58(6): 1192-1194.

Kosaka, K., H. Yamada, et al. (1998). "Comparison among the Methods for Hydrogen Peroxide Measurements To Evaluate Advanced Oxidation Processes: Application of a Spectrophotometric Method Using Copper(II) Ion and 2,9-Dimethyl-1, 10-phenanthroline." Environmental Science and Technology 32: 3821-3824.

Li, Ping, et al. (2005). "Synergistic antibacterial effects of β-lactam antibiotic combined with silver nanoparticles." Nanotecnology 16: 1912-1917.

Li, Xuan, et al. (2010). "Dissolution-Accompanied Aggregation Kinetics of Silver Nanoparticles."

Liu, Jingyu and Robert Hurt. (2010). "Ion Release Kinetics and Particle Persistence in Aqueous Nano-Silver Colloids." Environmental Science and Technology 44(6): 2169-2175.

McWilliams, Andrew. (2008). "Nanotechnology: A Realistic Market Assessment." Report Code: NAN031C.

Mock, J.J., M. Barbic, et al. (2002). "Shape effects in plasmon resonance of individual colloidal silver nanoparticles." Journal of Chemical Physics 116(15): 6755-6759.

Oberdorster, G., E. Oberdorster, et al. (2005). "Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles." Environmental Health Perspectives 11(7): 823-839.

Panacek, Ales., et al. (2006). "Silver Colloid Nanoparticles: Synthesis, Characterization, and their Antibacterial Activity." J. Phys. Chem. B 110(33): 16248-16253.

Soukupova, J., L. Kvitek, et al. (2008). "Comprehensive study on surfactant role on silver nanoparticles (NPs) prepared via modified Tollens process." Material Chemistry and Physics 3: 77-81.

US EPA (2008). Draft Nanomaterial Research Strategy (NRS). Washington, DC.

Wiesner, M.R., G.V. Lowry, et al. (2006). "Assessing the Risks of Manufactured Nanomaterials." Environmental Science and Technology 40: 4336-4345.